Review

The role of estrogens in cardiovascular disease in the aftermath of clinical trials

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ABSTRACT

The effects of estrogens on reproductive tissues and climacteric symptoms are unambiguous. However, their effects on other tissues and, in particular, the cardiovascular system remain controversial. In general, premenopausal women are protected from coronary heart disease (CHD) compared with aged-matched men but this 'female protection' appears to be lost after menopause, suggesting beneficial effects of female sex hormones on the cardiovascular system. This view has been supported by observational studies showing that estrogen replacement therapy (ERT) is associated with a 30% to 50% decrease in CHD risk in postmenopausal women. Nevertheless, randomized clinical trials in postmenopausal women, with or without pre-existing CHD, have found no benefits to combined hormone replacement therapy (HRT). A possible explanation for these apparently contradictory findings may relate to the divergent effects of estrogens depending on the state of the vascular endothelium. It has been suggested that estrogens may prevent the development and early progression of atherosclerosis by contributing to the maintenance of endothelial health but may have a neutral or negative effect on a dysfunctional endothelium or on advanced atheromatous lesions. Furthermore, it is possible that estrogens have adverse effects on other cardiovascular risk factors such as thrombosis and inflammation. It is also conceivable that a decline in vascular estrogen receptor expression with advancing age, through methylation of the estrogen receptor promoter, prevents postmenopausal women from deriving the beneficial cardiovascular effects of estrogens. In conclusion, estrogens possibly prevent the development of atherosclerosis through favourable effects on an intact endothelium, but once the vascular endothelium is damaged, the prothrombotic and possibly proinflammatory effects of estrogens are likely to predominate and prove harmful.

Key words: Estrogens, Hormone replacement therapy, Cardiovascular disease, Estrogen receptors

INTRODUCTION

Epidemiological data suggest that premenopausal

women are largely protected from coronary heart disease (CHD) compared with men of similar age[1]. This phenomenon, referred to as 'female advantage', is gradually lost after menopause, so that by the sixth decade women have the same incidence of CHD as men. The protection of premenopausal women from CHD has been attributed to the cardioprotective effect of female sex hormones[1]. Estrogens are involved in many physiological processes which are known to

be important for cardiovascular health in women and were until recently considered to protect women from cardiovascular disease (CVD). Extensive evidence from observational studies support this view, especially in regard to CHD[2,3]. However, randomized clinical trials in postmenopausal women, with or without CHD, have shown no benefits from combined hormone replacement therapy (HRT), casting doubt on the cardioprotective effect of estrogens on postmenopausal women[4,5].

It has recently been suggested that the loss of 'healthy vascular endothelium' may prevent women from deriving cardioprotective benefits from endogenous or exogenous estrogens[6]. According to this hypothesis, the favourable antiatherogenic and other favourable vascular effects of estrogens are endothelium-dependent and receptor-mediated. Consequently, endothelial injury or decline in vascular estrogen receptor expression can diminish the cardiovascular benefits of the sex hormones. This concept may in part explain the disparity between the observational studies showing a cardioprotective effect of estrogens in healthy postmenopausal women and the recently published clinical trials on secondary and primary prevention of CHD in postmenopausal women with HRT which showed a different effect[4,5].

In this review, we report on the biological effects of estrogens on the vascular system and in particular on endothelial function, and we analyze the divergent results from the observational studies and randomized clinical trials. It seems that the likely role of estrogens is to contribute to the maintenance of a healthy vascular endothelium but, in the presence of endothelial injury and the atheromatous vascular wall, the use of HRT could be harmful.

SOURCES OF ESTROGENS IN WOMEN

The naturally occurring estrogens 17β-estradiol (E2), estriol (E3) and estrone (E1) are C18 steroids derived from cholesterol through a series of enzymatic reactions in steroidogenic cells (Figure 1).

In women of reproductive age, estrogens are produced mainly in the ovaries. Under the influence of luteinizing hormone (LH) the ovarian theca cells produce two androgenic steroids, androstendione and testosterone, which are aromatized in granulosa cells into E1 and E2, respectively, by the action of follicle
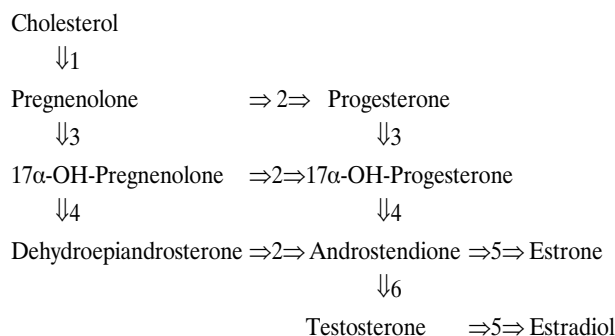
Cholesterol
$\Downarrow 1$

Pregnenolone $\Rightarrow 2 \Rightarrow$ Progesterone
$\Downarrow 3$ $\Downarrow 3$

17α-OH-Pregnenolone $\Rightarrow 2 \Rightarrow$ 17α-OH-Progesterone
$\Downarrow 4$ $\Downarrow 4$

Dehydroepiandrosterone $\Rightarrow 2 \Rightarrow$ Androstendione $\Rightarrow 5 \Rightarrow$ Estrone
$\Downarrow 6$

Testosterone $\Rightarrow 5 \Rightarrow$ Estradiol

Figure 1. Sex steroid biosynthetic pathways. Enzymes: 1: cholesterol-20 and 22-desmolase, 2: 3β-hydroxysteroid deydrogenase, 3: 17α-hydroxylase, 4: 17- and 20-desmolase, 5: aromatase, 6: 17-hydroxysteroid deydrogenase.

stimulating hormone (FSH). Following ovulation, the luteinized cells produce progesterone during the luteal phase of the menstrual cycle. Progesterone has two roles: transformation of the endometrium after estrogen priming (luteominetic effect), and antiestrogenic effect limiting proliferation of the endometrium.

In premenopausal women E2 levels range from 10-80pg/ml during the follicular phase and reach the level of 600pg/ml at midcycle. After menopause the concentration of E2 falls to 5-30pg/ml. The main estrogen in menopausal women is E1, resulting from aromatization in adipose tissue of androstenedione which is produced by the adrenals.

In the circulation, estrogens bind mainly to sex hormone binding globulin (SHBG) produced in the liver and only a small amount of estrogens (2-3%) remains free[7]. Changes in SHBG levels may influence the tissue availability of free estrogens but also of free androgens, since the latter are also bound to SHBG. Although estrogens themselves increase SHBG levels, androgens and high insulin levels have the opposite effect. In menopause, the fall of estrogens in women is associated with reduced SHBG leading to decreased binding and thus to an increase in the level of free androgens. Estrogens fall to a greater extent and the androgen/estrogen ratio rises. The alteration in the balance between androgens and estrogens may contribute to changes in body composition and other symptoms associated with the menopause[8].

In addition to endogenous estrogens, there are also exogenous sources of estrogen such as the oral contraceptives (OC) and hormone replacement therapy

(HRT). The OCs consist of ethinyl-estradiol combined with a synthetic progestogen. For HRT, conjugated equine estrogens (CEE) or other oral or transdermal forms of synthetic estrogens are used in association with an orally administered progestogen. The progestogen is administered either cyclically or in a continuous combined regimen and is included in order to avoid the risk of endometrial hyperplasia[9].

Selective estrogen receptor modulators (SERMs) belong to another category of chemical substances that have an estrogenic like activity with regard to vascular endothelium and bones, but an anti-estrogenic effect on the mammary gland and endometrium[10]. Tibolone is a new synthetic steroid with a progestogen-like structure that is converted to estrogenic and androgenic derivatives in different tissues and is also used as HRT[11]. Finally, phytoestrogens, derived from various plants, have an estrogen-like activity that may help in reducing climacteric and menopausal symptoms[12].

ESTROGEN RECEPTORS AND MOLECULAR EFFECTS

Estrogens act in target tissues by binding to estrogen receptors that belong to the large family of nuclear ligand-activated transcription factors[13]. Once bound by estrogen, the estrogen receptor undergoes a conformational change, allowing the receptor to bind with high affinity to specific DNA-sequences or response elements (EREs) and modulate transcription of target genes[14]. Two types of estrogen receptors are known; estrogen receptor-α (ERα) and estrogen receptor-β (ERβ), encoded by different genes. Both receptor types are found in the cells of the cardiovascular system as well as in various other human tissues. ERβ is distributed differently from ERα within the tissues of the body and probably mediates different cell functions, but this is a field that needs further investigation.

Several studies have demonstrated the presence of functional estrogen-receptors in vascular endothelial cells as well as in smooth muscle cells[15,16]. In women with premature atherosclerosis, a decreased ER expression on atherosclerotic arteries has been observed[17].

Regarding the role of ER in the regulation of endothelial function, a number of studies using selective ER-antagonists have taken place. The results of these studies have shown that 17β-estradiol enhances the production and activity of the enzyme nitric oxide synthase (eNOS), either via classical effects on gene transcription (genomic regulation), or via a non-genomic pathway[18]. The latter refers to a rapid stimulation of endothelial NO production in an ER-dependent manner[18,19]. There is strong evidence that eNOS is targeted to the endothelial plasma membrane, particularly to caveolae, which are specialized membrane domains[20-22]. A subpopulation of ERα is localized to endothelial-cell caveolae where they are coupled to eNOS in a functional complex that mediates non-genomic activation of eNOS. The rapid stimulation of eNOS by 17β-estradiol is due to an increase in intracellular calcium, mediated by tyrosine-kinase/MAP-kinase activation[23,24].

Since NO is the main vasodilator and the best antioxidant substance, its decreased production causes endothelial dysfunction. Thus, a reduced estrogenic action, either as a result of decreased estrogen levels or reduced number of functional ERα in blood vessels and endothelium, probably constitutes a considerable cardiovascular risk factor as it may lead to premature endothelial dysfunction and therefore to atherosclerosis[25].

VASCULAR EFFECTS OF ESTROGENS

The blood vessel wall consists of smooth muscle cells and a lining of endothelial cells, both of which have functional ERs as referred to above. Consequently, estrogens are believed to play a significant role with regard to vascular homeostasis. Their principal effect appears to be on the regulation of vascular tone. Generally, two types of factors modulate vascular tone: vaso-constrictive, such as endothelin-1, thromboxane, prostaglandin H2 and angiotensin, and vaso-relaxing factors. The predominant relaxing factor is NO, produced by the endothelial cells. Release of NO activates smooth muscle cell quanylate cyclase, which in turn causes an increase in cGMP production leading to vasodilation[26]. Other relaxing factors, such as prostacyclin and hyperpolarizing factor, act through cAMP and potassium channels. The NO release also has an anti-inflammatory and anti-atherogenic effect because it prevents platelet aggregation and suppresses smooth muscle cell proliferation[27].

These two actions prevent endothelial dysfunction

which is regarded as an early manifestation of atherosclerosis[28,29].

The clinical evaluation of endothelial function is based on the observation of vasodilative ability after pharmacological or mechanical stimuli[30]. For this purpose three techniques have been adopted. The first two exploit the administration of acetylcholine intracoronary and intraarterially, which provides a dilatory effect and an increase in blood-flow, respectively. The third technique uses increased blood-flow shear (flow-mediated) as a mechanical means to estimate vasodilation through NO release[30]. The most commonly used technique is measurement of branchial artery diameter changes with high-frequency ultrasound after blood pressure cuff-induced hyperemia[31]. The use of nitroglycerine (TNT) or nitroprusside helps to estimate the non-endothelium dependent vasodilation.

There is evidence that estrogens contribute to the maintenance of "endothelial health". In fact, the arterial endothelial NO seems to be the primary vascular target of estrogens[32] which exert two types of effects with regard to endothelial function.

First, estrogens have long-term effects which are mediated at the level of gene transcription (genomic regulation). These effects are due to an increase in the expression and activity of eNOS, as explained above[18,32,33]. Furthermore, it has been observed that estrogens (particularly unopposed CEE) reduce plasma levels of endogenous assymetric dimethylarginine (ADMA)[34], an inhibitor of eNOS[35] and a cardiovascular risk factor[36].

Clinical observations support these effects. Not only do estrogens cause vasodilation and increased blood-flow, but these responses also depend on plasma levels of estrogens. The observations that in young women endothelium-dependent vasodilation in branchial artery varies with the phase of the menstrual cycle, favours this conclusion[37,38]. Researchers have used blood pressure cuff-induced hyperemia to study endothelial function in premenopausal and postmenopausal women and found that there were greater vasodilatory responses in premenopausal women. When TNT or nitroprusside was used, the responses in pre- and postmenopausal women were quite similar[39-41]. Estrogen replacement therapy (ERT) given to postmenopausal women enhanced endothelium-dependent dilation in the branchial and coronary arteries[42,43].

Similar results were observed in women with premature ovarian failure or ovariectomy after the use of estrogen, as well as in young women who used oral contraceptives[41,44]. It is of interest that inclusion of progesterone in postmenopausal HRT may blunt the effects of estrogens on endothelial NO production[44].

In addition, estrogens exert short-term effects on the endothelium, causing rapid dilation by both endothelium-dependent and endothelium-independent pathways[33]. Thus, estrogens can cause rapid vasodilation (within minutes) when given intravenously or intraarterially in postmenopausal women[45]. These effects are probably mediated by a subpopulation of ERα localized to caveolae in endothelial cells and coupled to eNOS in a non genomic manner, as mentioned above[20-22].

Estrogens also exert additional effects. These include stimulation of prostacycline, that causes vasodilation, and inhibition of endothelin-1-production in human vascular endothelial cells[46]. In addition, estrogens prevent apoptosis in human vascular endothelial cells and inhibit the migration and proliferation of smooth muscle cells in vitro[47-49].

SYSTEMIC EFFECTS OF ESTROGENS

Effects on lipids and lipoproteins

Estrogens have beneficial effects on lipids and lipoproteins, but these effects depend on the type of estrogen used, the route of administration, the co-administration of progestogen and the type of progestogen[50].

In general, oral estrogens reduce cholesterol accumulation in peripheral tissues and increase its biliary excretion[51,52]. In particular, estrogens decrease total cholesterol and low-density-lipoprotein (LDL) cholesterol by about 5-15%, due to an increase in the liver LDL-receptors, and enhancement of LDL-catabolism and clearance. Estrogens also reduce apolipoprotein (a) {Lp (a)} and increase high-density-lipoprotein (HDL) cholesterol by about 10% by decreasing HDL-receptors in liver cells. On the other hand, estrogens increase plasma triglycerides (TG) by 20-25%.

In addition to its effect on serum lipid profile, estradiol has an antioxidant capacity so that when 17β-estradiol is given to menopausal women, it reduces

oxidation of LDL cholesterol and enhances endothelial NO bioactivity[53].

Transdermal estrogens do not affect serum lipids and lipoproteins and this is probably related to the absence of a first-pass hepatic effect[54]. On the other hand, progestogens have no influence on LDL or Lp(a), but they appear to have an antiestrogenic activity on HDL and TRGs[50].

Recent evidence suggests that remnant lipoprotein particles (RLPs) are the most atherogenic lipid particles. RLPs were associated with endothelial dysfunction and atherosclerosis and were identified as an independent risk factor for cardiovascular disease in women[55]. A recent randomized study has shown that HRT has beneficial effects on RLPs, with no influence on triglycerides[56].

Effects of estrogens on hemostasis

Hemostasis is a natural mechanism of the human body aimed at halting bleeding irrespective of its cause. For this purpose a number of coagulant factors play an important role. On the other hand, there are regulatory factors, i.e. anticoagulant factors and the fibrinolytic system, with opposite effects, so that hemostasis does not lead to vessel obstruction. An outline of this hemostatic balance is given in Figure 2.

Estrogens appear to interfere in the hemostatic balance as they regulate, through estrogen receptors, the expression of genes for a number of coagulant and fibrinolytic proteins[57]. This regulation is probably under genetic control because the hemostatic system of some women appears to be more sensitive than that of other women[58].

It has been reported that in postmenopausal women the levels of factor VII, factor vW and fibrinogen are elevated[59]. These factors are important risk markers for ischemic heart disease. On the other hand, the use of HRT in postmenopausal women decreases the level of fibrinogen, but also decreases protein S and antithrombin III and enhances activity of factor VII[60].

In addition, increased levels of PAI-1 have been found in postmenopausal women, leading to fibrinolysis inactivation that is associated with atherosclerosis[61]. Even small doses of ERT reduce PAI-1 and activate the fibrinolytic system. The combination with progestogens maintain the same beneficial effects, although transdermal estrogens do not influence PAI-
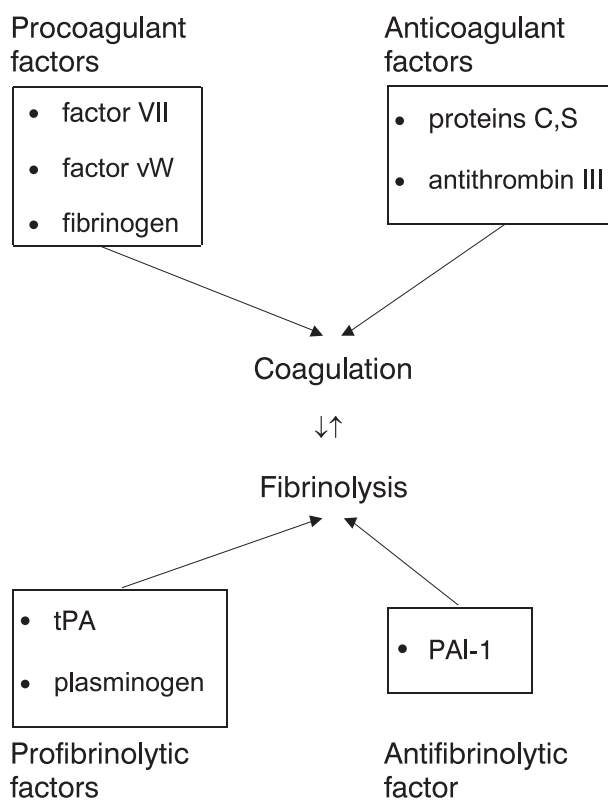
Figure 2. Hemostatic balance. Abbreviations: Factor vW:Factor von Willebrant, tPA:tissue plasminogen activator, PAI-1: Plasminogen activator inhibitor type 1.

1[62,63]. The activation of fibrinolysis does not appear to be dose-dependent, unlike the coagulatory activity[61].

Effects of estrogens on inflammatory markers

Atherogenesis and the ensuing plaque disruption are the result of an inflammatory process that takes place in the vessel wall. C-reactive protein (CRP) is the most important marker of inflammation and the strongest independent predictor of cardiovascular events in apparently healthy women[64,65].

There is evidence that the level of CRP rises in postmenopausal women using oral estrogens[66,67], although the latter reduce other inflammatory markers such as E-selectin VCAM-1, ICAM-1 and soluble thrombomodulin[68]. This indicates that the rise in CRP levels is not the result of an increased inflammatory response but has to do with a metabolic liver-activation. By contrast, studies in animals where estrogens were given subcutaneously or intravenously failed to demonstrate such pro-inflammatory effects[69]. It appears that the route of administration of estrogen may

be important with regard to its adverse effects on inflammatory markers[65].

Thus, the use of HRT in women with pre-existing coronary heart disease may become problematic[70]. HRT reduces serum levels of the cell adhesion molecules that may inhibit attachment of white blood cells to the vessel wall, but this therapy also causes a rise in serum levels of matrix metalloproteinase-9 (MMP-9). In addition, HRT decreases plasma levels of PAI-1 leading to plasmin activation, which may also activate metalloproteinases. These events in women with atheromatous plaques could result in digestion of the matrix proteins of the fibrous cap and therefore provoke thrombosis[71,72].

HORMONE REPLACEMENT THERAPY AND CARDIOVASCULAR DISEASE: CLINICAL STUDIES

Premenopausal women develop CVD less frequently than men of similar age. However, the protection that women appear to have during their reproductive age fades after menopause when the rate of coronary heart events tends to be equal to that of men[73]. Among the measures that have been taken over the last few decades to reduce the risk of CVD are lifestyle changes and pharmacologic interventions. HRT was among the pharmacologic interventions and was thought to have beneficial effects in postmenopausal women[74,75].

Observational studies

A large number of observational studies have suggested that the use of exogenous estrogens might be cardioprotective. In a review of population-based, case-control, cross-sectional and prospective studies of estrogen therapy (in the form of CEE), the relative risk for CHD was reduced by approximately 50%[76]. In current hormone users there were reductions in CHD-risk which ranged from 35% to 50%, compared to non-users[75,77]. In the Nurses' Health Study, in which 70,533 postmenopausal women were followed-up for 20 years, the relative risk of major coronary events was 0.61(CI: 0.52-0.71) for current use of estrogens, adjusted for age and the common cardiovascular risk factors[3]. In a recent study, the progress of coronary atherosclerosis was estimated by measurement of coronary artery calcium (CAC) with electron beam computed tomography (EBT)[78]. The participants of this study were 2,213 healthy postmenopausal women with a mean age of 59 years; half of the women were current users of HRT. The results showed that current HRT use decreased the possibility of high calcium score by 50%. Therefore, these results indicate that HRT use is associated with less coronary artery atherosclerosis as estimated by CAC, since high calcium scores are significant predictors of cardiovascular events in most large observational studies[79,80]. In addition, the study showed that the longer women received HRT, the greater was the fall in the calcium score (dose-response relationship).

In general, observational studies promoted the belief that HRT plays a cardioprotective role. However, in a recent observational study in women with established coronary heart disease, the use of HRT was harmful. There was an increase in the cardiovascular events in women who started HRT after a myocardial infarction[81].

With regard to the observational studies there are two important issues worthy of discussion. First there is the problem of bias. In these studies comparisons were made between women who have elected to take HRT and women who either elected not to take HRT or who themselves had not considered taking it (selection bias)[82]. These two groups of women may have had different lifestyles and educational or socioeconomic status. Women who used HRT were likely to have also taken more preventive health measures compared to women not on HRT, therefore, they were also likely to be at lower risk for CHD regardless of HRT use[83]. However, in the Nurses' Health Cohort Study, in which users and non-users had the same social status and educational level, hormone use also appeared to be protective[3].

The second issue concerns the type of hormone regimen used, as women in the observational studies used mainly unopposed CEE. On the other hand, it is known that progestogens may oppose some of the estrogen effects on the cardiovascular system[84]. However, a limited number of observational studies with combined HRT suggest that the effect of progestogen may not be substantial, although the type of progestogen used may be relevant.

As the role of HRT in menopause remained inconclusive, the need for clinical trials in order to clarify the potential role of HRT on cardiovascular disease in postmenopausal women became apparent.

Randomized clinical trials

The Heart and Estrogen/Progestin Replacement Study (HERS) was the first large clinical trial to test the use of HRT as secondary prevention in women with established coronary heart disease[4]. HERS was a double-blind, placebo controlled, randomized study of 2,763 postmenopausal American women, of mean age 66.7 years, who received combined continuous oral HRT {0.625mg CEE plus 2.5mg MPA (medroxyprogesterone-acetate) daily} or placebo. The trial was ended prematurely after 4.1 years because there was an increase in the coronary and thromboembolic events in women who used HRT during the first year, although the serum lipid profile had improved. By the fourth year of study, cardiovascular events were fewer in the HRT-users than in the placebo group. However, recently published data from an extension of the HERS study to 6 years (HERS II) have indicated that the trend towards reduction in cardiovascular events did not continue[85]. Because of an increase in the risk of venous thrombosis among HRT-users, it was suggested that HRT is not beneficial in women with established cardiovascular disease who already have atherosclerotic plaques prone to rupture.

There was some criticism of the HERS trial results[86]. The first criticism was the older age of women participating in the study. It was then suggested that lower doses of estrogens might be more beneficial with regard to the progress of atherosclerosis. Another issue was the fact that in HERS a specific combination of hormones was used (CEE+MPA). Whether the results would be the same if other combinations of estrogen+progestogen were used remained unanswered. The answer to this came from another study, the Papworth Hormone Replacement Therapy Atherosclerosis Study. In this study postmenopausal women used transdermal 17β-estradiol alone or in combination with norethisterone. Again, this regimen had no benefit in reducing CHD events in women with pre-existing CVD[87].

The second clinical trial was the Estrogen Replacement and Atherosclerosis (ERA), a randomized trial in postmenopausal women who had angiographically defined CHD at baseline in order to evaluate coronary artery changes after the use of HRT[88]. Again, neither CEE alone nor in combination with MPA were effective in slowing down the progress of atherosclerosis in coronary arteries, although beneficial effects

on lipid profile were noted.

It was concluded from these studies that HRT should not be used for secondary prevention of CHD. Subsequently, the role of HRT for primary prevention of CHD was addressed.

To this end, a large clinical trial, the Women's Health Initiative (WHI), was designed[5,89]. It was a randomized, double-blind, placebo-controlled, clinical trial of 16,608 postmenopausal women, aged 50 to 71 years (mean age 63.7 years), who received either continuous oral combined CEE (0.625mg) + MPA (2.5mg) or placebo daily. Another group of 10,739 postmenopausal women without uterus, as a result of hysterectomy, used CEE alone or placebo. This part of the study is still running. However, the first part was stopped after 5.2 years of follow-up because the risks exceeded the benefits. There was an increased risk of invasive breast cancer (8 cases/10,000 women), which rose the longer the women continued the therapy. The risk of non-fatal myocardial infarctions and coronary death also increased (7 cases/10,000 women) especially during the first year of the study. The risk of venous thromboembolism was doubled (18/10,000 women). Finally, there was an excess risk of stroke (8/10,000) throughout the trial. These results disappointed the optimists among the scientists who believed that HRT could prevent CVD in healthy postmenopausal women. On the other hand, some benefits from the use of HRT were identified. The incidence of hip fractures was reduced (8/10,000), as was the incidence of colorectal cancer (6/10,000). There was no overall benefit, however, over the 5.2 years of the trial.

The Estrogen in the Prevention of Atherosclerosis Trial (EPAT) followed up postmenopausal women without preexisting CHD in order to estimate the effect of the oral use of 1mg 17β-estradiol in the progress of carotid atherosclerosis[90]. Women in whom serum LDL was above 160mg/dl received antilipid therapy. The results showed that these women had no alterations in the carotid artery ultrasound after two years of oral therapy. In contrast, in women with low serum LDL-levels, the use of 17β-estradiol slowed the progression of carotid intima-media thickness in comparison to placebo. Overall, this trial showed a beneficial effect of estrogen use in healthy postmenopausal women with regard to atherosclerosis of the carotid arteries. On the other hand, another clinical trial,

the WELL-HART (Women's Estrogen/Progestin Lipid Lowering Hormone Atherosclerosis Regression Trial) in postmenopausal women with established coronary artery atherosclerosis, showed that the use of 17β-estradiol alone or in combination with MPA had no influence on the progression of atherosclerosis evaluated by coronary angiography, compared to placebo[91].

Surprisingly, therefore, the results from randomized clinical trials of HRT use in postmenopausal women for primary or secondary prevention of CHD do not support the observational data on the cardioprotective role of estrogens. Several possible explanations for the divergent data on postmenopausal HRT have been suggested.

The complexity of sex steroid effects

The main estrogen in the HRT used in the clinical trials is CEE which is usually prescribed in the U.S.A. On the other hand, 17β-estradiol is more commonly used in Europe, but there have been no observational data or large clinical trials for primary prevention with 17β-estradiol[86].

Another possible explanation might be the high dose of estrogens used. There is evidence that lower doses of estrogen have beneficial effects on lipoproteins and coagulant factors. Furthermore, older women, such as the participants in the two large clinical trials, may be more susceptible to inflammatory and thrombotic effects of higher doses of estrogen[92].

On the other hand, it is possible that the use of progestogen in combination with estrogen may mask estrogen's cardioprotective role[86]. This will probably be clarified at the end of the second part of the WHI study in which 10,739 women without uterus received CEE alone or placebo.

In addition, oral estrogens increase CRP[66,67] and induce expression of matrix metalloproteinases within the vessel wall. The latter can digest and weaken fibrous caps of vulnerable plaques, leading to thrombosis[71,72].

Another criticism of the WHI trial is the fact that the women participating were older, so that they were likely to already have existing atherosclerotic changes. Since atherosclerosis down-regulates estrogen receptors, the transcription of multiple genes, some of which are responsible for the potential protective effects of estrogen, is reduced and this fact cannot be excluded[86].

The state of the vascular endothelium

Experimental and clinical evidence suggests that the effects of estrogens are dependent on the integrity and functional status of the vascular endothelium and the presence or absence of atherosclerotic changes in the vascular wall. This 'healthy endothelium' hypothesis may in part explain the unfavourable findings of the HERS and WHI trials and guide future strategies of the use of HRT[6].

According to this hypothesis, endothelial injury or decline in vascular estrogen receptor expression can diminish the antiatherogenic properties of estrogens. Indeed, experimental studies have examined the effects of endothelial damage induced by balloon catheter injury in rabbits and how estrogens affect progression of atherosclerosis. It was found that the anti-atherogenic effects of estrogens were present, absent or reversed depending on the state of the arterial endothelium[93].

Studies in oophorectomized cynomolgous monkeys also support this hypothesis. Thus, in monkeys assigned to CEE (alone or in combination with MPA) beginning two years (approximately six human years) after oophorectomy and well after atherosclerosis was established, HRT had no effect on the extent of coronary artery plaque. However, when HRT was given to monkeys immediately after the oophorectomy, during the early stages of atherosclerosis, there was a 50% reduction in the extent of plaque formation[94]. In this regard, in the Nurses' Health Study women ranged in age from 30 to 55 years at enrollment and almost 80% began estrogens within two years after menopause[3]. In contrast, the mean age of the participants in the HERS and in the WHI trials were 67 and 63 years, respectively, and thus on average 10 years postmenopausal at the time of enrollment, an age at which atherosclerosis had already set in.

Furthermore, studies in humans have shown that non-diseased coronary artery vessels dilate in response to the administration of estrogens, whereas diseased vessels do not respond[95]. The lack of estrogen receptor expression in the presence of atherosclerosis may result in a decreased ability of vascular tissue to respond to estrogens[17]. In this regard, down-regulation of ERs, may have an impact on the effectiveness of

estrogen. It is well established that an important mechanism for down-regulation of gene-expression is methylation of a cytosine and guanine rich area in the promoter-region of the ERα-gene, called CpG-island. Methylation leads to permanent inactivation of gene-transcription in multiple systems[96].

It has been shown that age and perhaps vascular injury are associated with de novo methylation of the ERα-gene promoter in smooth muscle cells. These cells lose some of the normal growth control and, under certain conditions, proliferate abnormally and may contribute to the pathogenesis of the atheromatous plaque. Another scenario is the possibility that ERα-gene-methylation is the result of atherosclerosis and hyperproliferation[97].

It is tempting to speculate that in women with advanced atherosclerosis, ERα-gene methylation in diseased vessels may contribute to the lack of benefit after the use of HRT observed in the clinical trials with older postmenopausal women[97]. According to recent research evidence, methylation may possibly be reversed pharmacologically by the use of inhibitors of the DNA-Methyltransferase enzyme[98].

ALTERNATIVE THERAPIES TO HRT

Since the use of conventional HRT regimens has already been shown to have disadvantages, scientists have turned their interest to alternative therapies in order to minimize the side effects of estrogen and explore their beneficial effects.

The first category of these new therapies consists of the Selective Estrogen Receptor Modulators [SERMs], which are non-steroidal estrogen compounds with an estrogen like effect (on lipoproteins and bones) as well as an estrogenic antagonist effect on the mammary gland and endometrium[7]. Raloxifene reduces the level of LDL-cholesterol and fibrinogen in plasma, but has no effect on HDL levels[99]. There have been various studies with divergent results. In ovariectomized cholesterol-fed rabbits, a reduction in aortic atherosclerosis was reported with the use of raloxifene but to a lesser extent than that with the use of 17β-estradiol[100]. Studies in monkeys showed no beneficial effect of raloxifene[99]. In the MORE trial (Multiple Outcomes of Raloxifene Evaluation Trial), with more than 7,000 postmenopausal women participating, raloxifene was shown to have no benefits compared with placebo in regard to CHD and cardiovascular events, but this was not the primary aim of the study. However, in women with increased risk of cardiovascular disease there were fewer events compared with placebo[101]. Another study, the RUTH trial (Raloxifene Use for The Heart Study), is currently testing the impact of raloxifene on cardiovascular end-points in postmenopausal women. The results of this trial will provide further information on the role of SERMs in CVD.

Another category, with tibolone as first representative, is the selective tissue estrogenic activity regulators (STEAR). STEAR have a progestogen-like structure that is converted to estrogenic and androgenic derivatives in-vivo. Tibolone is effective in the control of climacteric symptoms (due to its 3-OH metabolites) and in maintaining bone density, without stimulating tissues such as the endometrium and mammary gland[102,103]. Thus tibolone is different from estrogens and SERMs as it reduces menopausal symptoms and the risk of thromboembolism (it promotes fibrinolysis rather than thrombosis). Tibolone also decreases not only triglycerides and Lp (a) levels but also HDL-cholesterol[104,105]. However, there have been no clinical trials on the effect of tibolone on the primary prevention of cardiovascular disease in postmenopausal women.

Finally, within the spectrum of alternative therapies to HRT are the phytoestrogens, a group of natural compounds derived from herbs with estrogen agonist and antagonist properties[106]. The strongest estrogenic activity has been observed in soy in which the removal of a glucone-group significantly raises its bioactivity[106-110]. High estrogen activity was also discovered in extract of fo-ti, not previously reported[106]. Soy phytoestrogens have shown beneficial effects on endothelium-dependent vasodilation in the development of atherosclerosis in non-human primates[108,111]. Some studies in postmenopausal women, though not all, have shown beneficial effects on lipid profile and in endothelial function[112]. Clinical end-point data are not available, however, so that recommendations regarding the use of phytoestrogens for the prevention of CVD cannot be formulated as yet.

CONCLUSIONS AND FUTURE DIRECTIONS

Based on the favourable effects of estrogens on various tissues and functions, including the cardiovas-

cular system, clinicians hoped that the use of estrogen alone or in combination with progestogen as replacement therapy in postmenopausal women would offer, among other benefits, cardioprotection. Unfortunately, clinical trials have not confirmed the cardioprotective role of HRT. It appears that in postmenopausal women with established coronary heart disease, HRT use might in fact be harmful because it increases cardiovascular events and the risk of venous thrombosis. Possible explanations for the divergent data between the observational studies and clinical trials include the type of estrogen and the high doses used, the possible antagonistic effects of progestogen, the age of the women who participated in the clinical trials and the state of the endothelium and stage of atherosclerosis at the time of intervention.

The early danger for both coronary heart disease and stroke observed in both the HERS and WHI trials may be a thrombotic effect, reflecting a synergistic effect between one of the relatively common hypercoaculable states and hormone therapy[113]. Another possible explanation is the pro-inflammatory effect of oral estrogens, with rupture of vulnerable plaques[86].

Although clinical trials showed not only lack of benefits but also an adverse effect on the cardiovascular system after the use of HRT, one cannot ignore studies showing the beneficial effect of estrogens in the early stages of atherogenesis during the menopausal transition and the early years after menopause. In the ensuing years new strategies should be adopted in clinical trials. Thus, the use of lower doses of estrogen or combinations with different progestogen, novel estrogen agonists including vascular SERMs, a combination of SERMs or phytoestrogens with low-dose estrogens and better timing of intervention are possibly some of the future directions for Hormone Replacement Therapy.

On the other hand, it is important to emphasize behaviour changes and, where appropriate, lipid and blood pressure medication, which have been proved to reduce CHD-risk in postmenopausal women, should be considered[86]. For the time being, however, HRT is suspended for the primary and secondary prevention of CHD in postmenopausal women.

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